methods of production and quality of silica are unlikely, a process of refinement can be expected, leading to even better deactivated materials.

While it would be encouraging to believe that the benefits of some of the other approaches will be recognized and exploited, the conservative nature of the pharmaceutical analyst – partially driven by the heavily regulated nature of the industry – and the dominance of reversed-phase methods make this unlikely.

See also: **II / Chromatography : Liquid :** Column Technology; Ion Pair Liquid Chromatography; Mechanisms: Ion Chromatography; Mechanisms; Normal Phase; Mechanisms: Reversed Phases. **III / Porous Polymers : Liquid Chromatography**.

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DropBooks

Capillary Electrophoresis

K. D. Altria and S. M. Bryant, GlaxoWellcome Research Centre, Hertfordshire, UK

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Introduction

In recent years the analysis of pharmaceuticals has been predominantly performed by high performance liquid chromatography (HPLC), which offers a number of advantages over other alternative techniques. These advantages include automated and precise sample injection devices, sensitive detection and high capacity autosamplers. HPLC is supported by other techniques such as thin-layer chromatography (TLC) and gas chromatography (GC). In the late 1980s a further technique, that of capillary electrophoresis (CE), became recognized as a viable alternative and complementary technique to HPLC. Modern CE instruments offer some of the same features as HPLC in terms of automation and autosampler capacity, although precision and sensitivity are not as good.

The wide range of application areas for CE within pharmaceutical analysis mirror well those of HPLC. These areas include the determination of drug-related impurities, chiral separations, main peak assay, stoichiometric determinations and the analysis of vitamins. The majority of pharmaceuticals are synthetic organic molecules that are well suited to analysis by HPLC or CE. There is an increasing move in pharmaceutical companies to the development of new pharmaceuticals that are based on biomolecules such as peptides and DNA. Traditionally these biomolecules have been analysed using electrophoretic techniques; for this reason CE has been widely applied to the analysis of biomolecule pharmaceuticals.

One of the attractive features of CE is that method development can be relatively simple for uncomplicated separations of ionizable pharmaceuticals. For example the majority of pharmaceuticals are basic drug salts. Use of a low pH electrolyte causes these basic drugs to protonate and become cations, and thus allows separation by CE. There are also a number of drugs with acidic functionalities that can

be separated by CE as anions using high-pH electrolytes. Neutral solutes require the use of micellar electrokinetic chromatography (MEKC) methods. Water-insoluble drugs can be separated in CE using traditional aqueous-based electrolytes but there is an increasing tendency towards the use of nonaqueous solvent systems in CE. The ease of method development is also a key feature in the use of CE for chiral separations, as a range of chiral additives can be quickly and effectively assessed in automated unattended injection sequences.

The use of indirect UV detection for the detection of small inorganic and/or organic cations and anions is widespread. These applications include determination of metal ion contents and inorganic anions such as sulfate and chloride. Traditionally determination of these species is performed by ion exchange chromatography but CE offers specific advantages in terms of ease of operation and reduced time and cost of consumables.

In this article the various application areas of CE in drug analysis are covered with some illustrative examples of reported applications. The discussion also includes some details regarding the analytical performance levels described for these analyses.

Determination of Drug-related Impurities

Capillary electrophoresis is increasingly being viewed as an alternative, complementary technique to HPLC for the determination of drug-related impurities. A number of applications have been reported, many with detection of the impurities at the 0.1% level or lower. Often low UV wavelengths (190–200 nm) are used to improve the detection limits obtained. The ability of CE to give a different selectivity to HPLC and/or TLC provides a further means to characterize the impurity content and profiles in drugs.

The separation of structurally similar drug-related impurities is difficult as the drugs and the related impurities often have very similar electrophoretic mobilities. Therefore the separation conditions must be optimized to enable a good resolution to be obtained, especially as low detection limits are generally required for the related impurities in the presence of a large drug peak. The pH of the separation is the most important optimization factor for separation of ionic species. However, the concentration and type of surfactant used is the most important factor for resolution of neutral and/or charged species by MEKC. Other factors that can be optimized in method development include the addition of ion-pair reagents and cyclodextrin and the ionic strength and type of electrolyte. These factors influence the shape of the main peak and appropriate optimization may allow resolution of a closely exiting impurity. For instance, various types and concentrations of ion-pair reagent have been employed in conjunction with cyclodextrin in the optimization of the separation of remoxipride and related impurities. **Figure 1** shows the separation achieved of remoxipride and eight related impurities using a relatively complicated pH 3 phosphate buffer which contained 40 mM hydroxypropyl beta cyclodextrin and 20 mM tetrabutylammonium bromide.

Many examples of the use of CE for the analysis of drug-related impurities have been published that have shown CE to be a useful complementary technique to support HPLC. These methods have been validated and are in routine use in many pharmaceutical companies. These include a CE method that has been used to monitor the stability of a cephalosporin in solution. Validation included specificity, linearity and repeatability by different analysts on different days. Migration time precision was less than 1% RSD (relative standard deviation). Injections were performed from sample solutions every 30 min to monitor the solution stability online. The cephalosporin (Roche compound RO 23-9424) was found to be twice as stable in an L-arginine/sodium benzoate/saline solution than when prepared in water.

Chiral Separations

Undoubtedly the most frequently used selectors in free solution CE are cyclodextrins (CDs). Other possibilities include crown ethers, carbohydrates, proteins and chiral antibiotics. The majority of pharmaceutical applications have involved the use of CDs. The most widely used approaches in MEKC are mixtures of sodium dodecyl sulfate (SDS) and CDs or bile salts, which are naturally occurring chiral surfactants.

There have been many quantitative applications of CE to the separation of drug enantiomers and these have included several reports of the validation of these methods. Method validation for chiral CE methods is similar to that undertaken for validation of an HPLC method. In the validation of a CE method, aspects such as the precision of injection, detection limits, and method repeatability on different capillaries using different operators and reagents are important. As in other separative techniques, comigration of related impurities with either of the enantiomer peaks is possible. Therefore it is necessary to establish the migration position of all available related substances in method selectivity studies. This procedure has been performed in a number of chiral CE methods including the chiral separation of a cholesterol-lowering agent (**Figure 2**). The ability of the

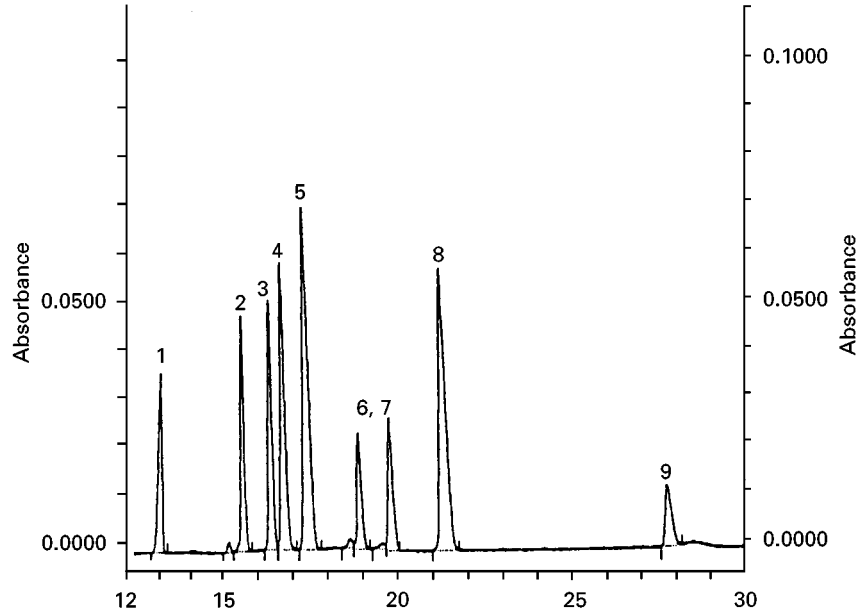

Figure 1 Effect on resolution of remoxipride analogues by variation of the tetrabutylammonium ion (TBA^+) concentration (0.1 mmol L^{-1}) in the presence of hydroxypropyl-β-cyclodextrin (HP-β-CD; 40 mmol L^{-1}) using a phosphate buffer at pH 3.0. The TBA^+ concentrations were (A) 10 mmol L^{-1} and (B) 20 mmol L^{-1}. The peaks are: 1, FLA 708; 2, FLA 739; 3, FLA 83; 4, FLB 526; 5, FLA 731 (remoxipride); 6, NCR 513; 8 = FLA 740; and 9, FLA 797 (x-scale in min). (Reproduced from Stalberg O, Brotell H and Westerlund D (1995) *Chromatographia* 40: 697–704, with permission from Elsevier Science.)

method to quantify accurately the enantiomeric impurity is also an important part of method validation and is often demonstrated by recovery experiments in which accurately known amounts of the impurity are spiked into standards of reference material. **Table 1** shows recovery data obtained during validation of a method for enantio-purity determination of a cholesterol-lowering drug.

Main Component Assay

The use of CE for main component assay is possible as commercially available instruments offer a high degree of automation and are capable of unattended injection sequences by use of PC-controlled autosamplers. The injection precision demands in many pharmaceutical companies is stringent for separative

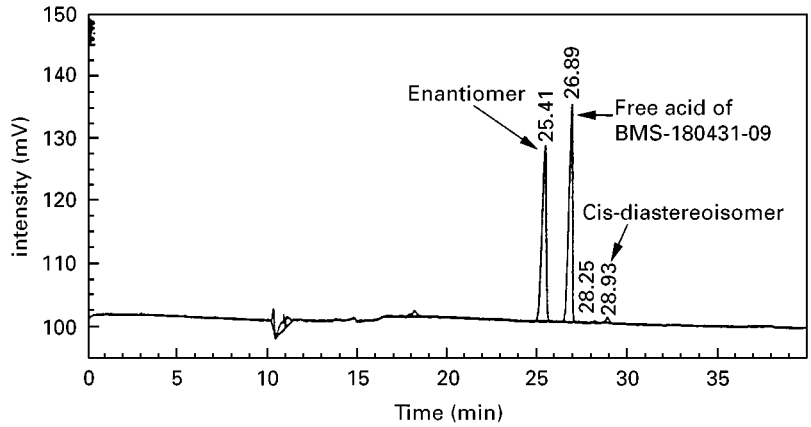

Figure 2 CD-MEKC of a racemic mixture of BMS-180431-09 with some trace *cis*-diastereoisomer added. Conditions: 0.01 mol L^{-1} (1.5 g per 100 mL) hydroxylpropyl-β-cyclodextrin, 0.1 mol L^{-1} sodium borate, 0.03 mol L^{-1} SDS, pH 9.3, 20 kV, current $\sim$ 65 μA, 50 cm effective capillary length, 50 μm capillary width, UV adsorption detection at 200 nm; sample was 0.3 mg mL^{-1} in water. (Reproduced with kind permission from Noroski JE, Mayo DJ and Moran M (1995) *Journal of Pharmaceutical and Biomedical Analysis* 13: 45–52.)

Table 1 Percentage recovery of enantiomer added to BMS-180431-09

Spike (% w/w)	*Found*	*Recovery*
0.36	0.37	102.7
0.65	0.65	100.5
0.80	0.84	104.6
1.10	1.11	100.9

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techniques such as HPLC and CE and figures such as 1–1.5% RSD are common practice. This can be routinely obtained by HPLC but this is not always the case for CE as the injection volumes are very small (1–20 nL) and can be variable. The use of internal standards to eliminate injection volume-related errors can dramatically improve the precision in CE and allow the required precision requirements to be achieved. For example internal standards were used in the validation of a simple pH 2.5, 25 mmol L^{-1} phosphate buffer for assay of a wide range of basic drugs. The method was shown to have good sensitivity, linearity (correlations > 0.999) with RSD values of 0.3–2.0% for peak area ratios. The robustness of the method to deviations in the method settings was satisfactorily assessed using an experimental design. Shelf-lives of electrolyte and sample solutions were assessed (3 months and 14 days, respectively). Good precision was obtained using either aminobenzoic acid or imidazole as the internal standard. Good agreement between CE data and the label claim for tablets was obtained (**Table 2**).

An MEKC method was successfully validated according to US Pharmacopoeia guidelines for analysis of hydrochlorothiazide and chlorothiazide. The method involved use of a 20 mmol L^{-1} borate buffer containing 30 mmol L^{-1} SDS. A 100 μm capillary was employed to give large peak areas in order to minimize integration-related errors. Careful control of the method settings such as injection time, temperature and sample concentration enabled values below 1% RSD to be routinely obtained with no internal standard. Recoveries ranged from 99.5–100.6% absorbance unit full scale (AUFS) for hydrochlorothiazide over the range 50–150% of target concentration. Detector linearities were greater than 0.998. Analyses were successfully repeated on different days by different analysts using different capillaries to demonstrate robustness.

Table 2 Assay results by CE for tablets and drug substance

Label claim	*Analysis*	
Lamiduvine content (mg per table)		
150	HPLC	152.2
	CE	155.6
100	CE	104.0
Histamine acid content (% w/w)		
	CE	
	Sample 1	100.3, 100.2
	Sample 2	100.8, 100.7
	Average	100.5

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Stoichiometric Determinations by CE

During development of a new drug, a range of different salts may be synthesized to compare pharmaceutical properties such as solubility, stability and crystallinity. The ratio of the drug to counterion is known as the drug stoichiometry and this needs to be characterized analytically. The typical stoichiometry is a 1 : 1 drug : counterion mixture; however, 2 : 1 and 1 : 2 compositions are frequently manufactured, depending upon the ionic nature of the drug and/or counterion. There is a clear analytical need to quantify drug : counterion levels to demonstrate that the correct salt version has been manufactured and that the required stoichiometry can be reliably achieved batch-to-batch when the final drug salt has been selected.

The counterion of basic drugs include inorganic ions such as sulfate and chloride or organic ions such as maleate, fumarate, acetate or succinate. Cations analysed involve a range of metal ions including Na^+, K^+, Mg^{2+}, Ca^{2+} and simple low molecular weight amines. Since these analytes possess little or no chromophore, indirect UV detection is generally necessary. However, some larger anionic counterions such as benzoates and simple organic acids can possess sufficient UV absorption to allow direct UV detection. Alternatively, metal ions may be complexed on-capillary to form metal chelates, which can then be detected by direct UV measurement.

The quantitative aspects of a CE method for the determination of calcium in calcium acamprosate has been validated. Standard solutions of calcium carbonate and test solutions of calcium acamprosate containing 100 ppm of Ca^{2+} and Mg^{2+} (internal standard) were determined by indirect detection at 214 nm. **Table 3** shows the determination of calcium in calcium acamprosate and the results compare favourably with the theoretical content.

Table 3 Comparison of CE and titration results for the calcium content in calcium acamprosate drug substance

Batch	*Theoretical content*	*CZE*	*EDTA titration*
C110	10.01% m/m	10.01% m/m (10.00, 10.04)	9.98% mass/mass
OTA 37	10.01% m/m	9.98% m/m (9.96, 10.01)	10.08% mass/mass

CZE, capillary zone electrophoresis; EDTA, ethylene diaminetetracetic acid; % m/m. Reproduced from Fabre H, Blanchin MD, Julien E *et al.* (1997) *Journal of Chromatography A* 772: 265–269, with permission from Elsevier Science.

Table 4 Comparison of CE and microanalysis/theoretical results for chloride and sulfate content

Batch		*Theoretical concentration*	*Microanalysis*	*CE results*
Chloride (% w/w)				
GRD1	A	8.0	–	8.0, 7.9
GRD2	A	9.6	9.5	9.3, 9.3
	B	9.6	9.6	9.4, 9.4
	C	9.6	9.5	9.2, 9.7
	D	9.6	9.4	9.9, 9.6
	E	9.6	9.5	9.3, 9.5
Sulfate (% w/w)				
GRD3	A day 1	16.6		16.7
	A day 2	16.6		16.8

GRD, Glaxo Research and Development. Reproduced with kind permission from Altria KD, Goodall DM and Rogan MM (1994) *Chromatographia* 38: 637–642.

Levels of chloride and sulfate have been determined using electrolyte containing chromate and tetradecyltrimethylammonium bromide (TTAB). Sample solutions were prepared to give 100 ppm of chloride as sulfate as appropriate. AnalaR grade salts such as NaCl were used as reference standards. **Table 4** shows the good agreement between the average CE results, microanalysis data and the theoretical content. Peak areas were used to calculate % w/w in samples of three different drug substances. Improved data for injection have been obtained using an internal standard. A detection limit of 0.5 μg mL^{-1} was reported for standard anions.

Separation and detection of a range of organic acids is possible using phthalate as the background absorber (**Figure 3**). The method has been validated for the quantitation of both succinate and maleate content in drug substance batches.

Vitamin Analysis

The majority of vitamin determinations are currently performed by HPLC with UV detection. These HPLC methods involve gradient elution and often extensive sample work-up prior to analysis to remove matrix interferences. The majority of vitamins are water-soluble acidic compounds and can be readily determined by free solution CE using high pH electrolytes. Water-insoluble and neutral vitamins require the use of MEKC. Many of the vitamins are acids or contain groups that ionize at high pH and therefore borate or phosphate buffers in the pH range 7–9 have been used extensively.

The CE and MEKC methods have been compared with the USP HPLC method to determine various B group vitamins in capsules, tablets and syrups. **Table 5** shows the data obtained by the three techniques to be equivalent. The use of an internal standard (paracetamol) improved the CE and MEKC precision from 7–10% RSD to 1%. Accuracy and repeatability of the MEKC method was demonstrated by spiking appropriate levels of the vitamins into artificially prepared mixtures of the tablet excipients.

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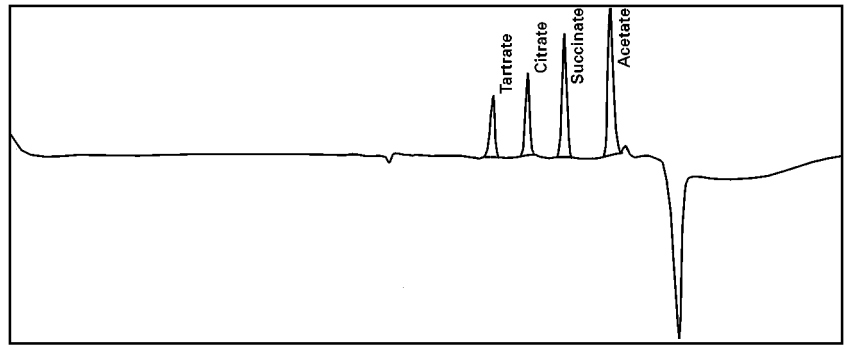

Figure 3 Separation of a selection of organic acids using phthalate as the background absorber. Separation conditions: fused silica capillary 27 cm × 75 μm; buffer, 50 mmol L^{-1}, 4-morpholineethanesulfonic acid (Mes), 0.5 mmol L^{-1} TTAB and 5 mmol L^{-1} phthalate adjusted to pH 5.2; voltage − 3.0 kV; wavelength 254 nm indirect detection (detector signal reversed); temperature 30°C. (Reproduced with kind permission from Altria KD, Assi KH, Bryant SM and Clark BJ (1997) *Chromatographia* 44: 367–371.)

Table 5 Cross-validation of vitamin assay results by CE, MEKC and HPLC

Sample	*Analyte*	*Results as % label claim*		
		CE	*MEKC*	*HPLC*
Tablet	B_1 (15 mg)	118.7 ± 1.7	123.6 ± 2.6	123.8 ± 3.6
	PP (50 mg)	110.8 ± 3.1	108.0 ± 1.2	108.7 ± 2.1
	B_2 (15 mg)	99.0 ± 2.2	99.4 ± 2.1	103.9 ± 0.7
	B_6 (10 mg)	110.9 ± 3.3	113.7 ± 1.7	112.4 ± 3.2
Syrup (5 mL)	B_1 (10 mg)	117.2 ± 4.0	112.4 ± 1.3	111.6 ± 1.6
	PP (20 mg)	111.2 ± 1.4	109.4 ± 0.9	111.5 ± 3.4
	B_2 (1 mg)	115.4 ± 1.5	119.3 ± 2.9	117.2 ± 2.2
	B_6 (5 mg)	109.9 ± 1.4	106.2 ± 3.1	113.2 ± 3.9
Soft capsule	B_1 (10 mg)	122.0 ± 2.2	126.6 ± 1.7	n/a
	PP (30 mg)	111.3 ± 1.8	108.6 ± 1.7	n/a
	B_2 (7 mg)	112.1 ± 3.1	114.9 ± 1.6	n/a
	B_6 (5 mg)	108.6 ± 1.8	108.2 ± 1.6	n/a

n/a, not analysed. Reproduced with permission from Boonkerd S, Detaevernier MR and Michotte Y (1994) *Journal of Chromatography A* 670: 209–214.

Six replicate samples were analysed on each of four separate days and acceptable data were obtained for average recovery and precision from the pooled assay results.

Figure 4 shows the separation of a range of common vitamins in a pharmaceutical preparation. The components are separated using a micellar electrolyte containing the surfactant SDS with acetonitrile and cyclodextrin modifiers. Detection is at 200 nm.

Levels of vitamins B_1, B_3, B_6, B_2, and C in injection solutions have been determined using an MEKC method with ethylaminobenzoate as an internal standard to give RSD values for precision of less than 2%. Agreement with label claims ranged from 98.8% to 104% for the five components.

Regulatory Aspects

The acceptance of CE methods by regulatory authorities has been highlighted in a confidential survey of a number of major UK and US pharmaceutical companies. All CE methods submitted had been accepted without technical query. The companies surveyed indicated that they had no reluctance to submit appropriate CE data in submissions. The survey was conduced in mid 1994 and the number of submitted methods has certainly increased since then. For example, a stability indicating MEKC method for the analysis of BMS-188484 has been successfully included in a regulatory submission from Bristol-Myers-Squibb.

Pharmacopoeia have also recognized the advancing application of CE within pharmaceutical companies

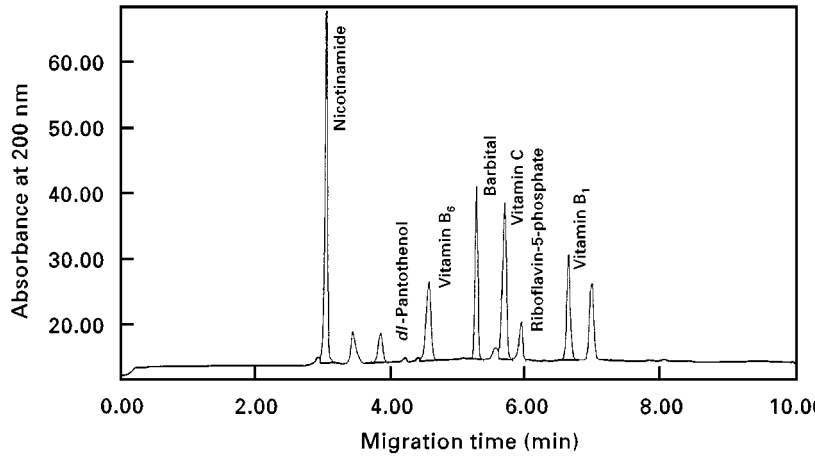

Figure 4 Separation of a range of vitamins.

and a draft USP general chapter on CE has been published (*Pharm Forum* 1996) in anticipation of future monographs containing CE analytical methods.

Conclusions

The use of CE in the analysis of pharmaceuticals is now becoming firmly established as a useful complement and alternative to the more widely employed technique of HPLC. The major attractions of CE are that considerable cost and time savings are possible, especially in the areas of chiral analysis and in the determination of solutes having limited or no chromophore. The recent advent of the use of nonaqueous solvents in CE should extend further the application range of the technique. Routine CE methods have been established in many industrial laboratories and CE methods have been successfully submitted to regulatory authorities.

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DropBooks

Chiral Separations: Liquid Chromatography

W. J. Lough, University of Sunderland, Sunderland, UK

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Introduction

Between the mid-1980s and the mid-1990s great advances were made in the development of commercially-available methodologies for the separation of enantiomers. These developments were undoubtedly catalysed by the need to determine the enantiomer content of chiral drug substances, chiral drug products and samples of chiral drugs in biological fluids in the pharmaceutical research and development environment. It had long been recognized that, since it is possible to distinguish between enantiomers in a chiral environment, enantiomers might be expected to have different effects on the body which contains potential chiral 'selectors' such as proteins, peptides, carbohydrates and enzymes. The action of a drug on the body or of the body on the drug involves many processes including the following:

- active transport
- plasma binding
- tissue binding
- receptor protein binding
- drug metabolism
- active secretion.

These and many of the other processes involve or may involve mediation by a protein or enzyme so that there is no shortage of opportunities for chiral discrimination to take place. As a consequence it is actually quite rare for enantiomers to have very similar pharmacological and toxicological properties. They must therefore be treated as if they were different drugs, and their use in combination in a drug product in a fixed 50 : 50 ratio as the racemate must be justified to the governmental regulatory bodies which issue licences to companies to produce and market drugs. Not only is this usually not justifiable,